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Ana Filipa da Silva Ferreira

Uma revisão sistemática sobre monitorização terapêutica do infliximab e adalimumab: níveis, resultados clínicos e ensaios

A systematic review on infliximab and adalimumab drug monitoring: levels, clinical outcomes and assays

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A Systematic Review on Infliximab and Adalimumab Drug Monitoring: Levels, Clinical Outcomes and Assays

ORIENTADOR

Professor Doutor Fernando Magro

COORIENTADOR (se aplicável)

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Faculdade de Medicina da Universidade do Porto, 11 / 03 / 2017

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A Systematic Review on Infliximab and Adalimumab Drug Monitoring: Levels, Clinical Outcomes and Assays

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Background: Immunogenicity to therapeutic proteins has been linked to loss of response by a large percentage of patients taking anti–tumor necrosis factor-alpha agents. Drug monitoring can be extremely useful, allowing physicians to adjust the therapeutic scheme individually. This article aims to systematically review the published data with respect to cutoff levels of infliximab (IFX) and adalimumab (ADA) and relate them to the methodology adopted for quantification of IFX and ADA levels and clinical outcomes.

Methods: The PubMed database was searched to identify studies focusing on the association between IFX or ADA cutoff levels and clinical outcomes in patients with inflammatory bowel disease.

Results: Of the 1654 articles initially selected by queries, 20 were included. A receiver operating characteristic curve analysis was performed to identify cutoff levels of IFX or ADA that correlated with a clinical outcome, but only 6 studies performed the same analysis for antidrug antibody levels. Cutoff levels were different between studies. The methodology chosen for level quantifications, clinical outcomes, and sample size and characteristics were also different. Nevertheless, measurement of drug levels should be performed during maintenance, and with loss of response, with persistent high levels of C-reactive protein, and when mucosal lesions are still present. In these scenarios, drug and antidrug levels were correlated with clinical outcomes.

Conclusions: Concerning drug levels monitoring any methodology is adequate. With respect to antidrug antibody levels, it will be necessary to define a gold standard method or to establish different cutoff levels for different methodologies.

(Inflamm Bowel Dis 2016;0:1–13)

Key Words: anti-infliximab antibodies, clinical outcomes, infliximab trough levels, therapeutic drug monitoring

Infliximab (IFX) and adalimumab (ADA) are antitumor necrosis factor-alpha (TNFα) agents that have changed the clinical course of many autoimmune diseases such as inflammatory bowel disease (IBD), psoriasis, and rheumatoid arthritis. These agents have been successfully used in the past decades to treat patients with IBD, even in those who were refractory to conventional therapy.[1–5] Introduction of these agents to the drug market allowed physicians to aim for more than clinical remission, as these new drugs were proven to induce endoscopic remission and mucosa healing in patients with either Crohn's disease (CD) or ulcerative colitis (UC).[6–8] Despite this, up to 70% of patients lose responsiveness over time.[9] Many mechanisms may be involved in the loss of response, but immunogenicity to the antibody itself is so far the best studied.[10] The presence of antibodies to IFX (ATIs) in patients' serum was associated with a 3-fold higher risk of loss of response than in patients who did not have ATIs in their serum.[9] Although ADA is a fully human monoclonal antibody drug, immunogenicity to this drug has already been described and a negative correlation between the presence of antibodies to ADA (ATA) and ADA trough levels (TLs) was demonstrated.[11] However, the influence of ADA levels in clinical and endoscopic remission is not well established yet.

When patients lose response to anti-TNFα agents, their physicians have roughly 4 options: (1) dose escalation, (2) addition of an immunomodulator, (3) change to another class of drugs, or (4) change to another anti-TNF agent.[12–17] Currently, physicians have to empirically decide since measurement of drug and antidrug antibody levels is not yet used in daily practice. Many authors have highlighted the importance of knowing drug and antidrug antibody levels to better adjust the therapeutic scheme.

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F. S. Ferreira and J. Afonso contributed equally to the article. F. S. Ferreira and J. Afonso were involved in data collection and interpretation, and drafting the manuscript. P. Pinto-Lopes was involved in the data interpretation. F. Magro was involved in the conception and design of the study, interpretation of data, and drafting and revision of the manuscript. All authors read and approved the final manuscript.

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Nonetheless, most authors emphasize the need to find a valid assay, especially to measure antidrug antibodies and to set cutoff levels to help in decision-making.[18–20] The aim of this article was to systematically review the published data with respect to IFX and ADA levels, the methodology applied, and the relationship with clinical outcomes.

MATERIALS AND METHODS

A systematic review focusing on the association between IFX, ADA TL, ATIs, ATAs, and clinical outcomes in patients with IBD was performed.

Search Strategy

A literature search was performed, through July 2015, using the PubMed database with the following keywords and Medical Subject Headings (MeSH) terms: "{(adalimumab[All fields]) OR (infliximab[All fields])} AND {(inflammatory bowel disease [MeSH Terms]) OR (inflammatory bowel diseases[MeSH Terms]) OR (crohn's disease[MeSH Terms]) OR (colitis, ulcerative[MeSH Terms]) OR (crohn disease[MeSH Terms])} AND ([clinical response] OR [clinical remission] OR [disease activity] OR [clinical outcomes])." Considering this is a hot topic, we decided, on December 2015, to perform an additional literature search on abstracts presented on 3 reference congresses. The European Crohn's and Colitis Organisation (ECCO) Website was searched for all published abstracts related with this topic, using the terms "infliximab ifx" and "adalimumab ada"; The United European Gastroenterology Week (UEGW) Website was searched for abstracts from the last United European Gastroenterology week in Barcelona; The Digestive Disease Week (DDW) Website was searched for abstracts from the past 5 years, using the terms "infliximab levels" and "adalimumab levels" in title, abstract, or keywords.

Eligibility Criteria

The inclusion criteria were: (1) articles studying the association between IFX or ADA cutoff levels and clinical outcomes in patients with IBD and (2) articles written in English.

We excluded studies that (1) were systematic reviews, (2) used another anti-TNF-α agent rather than IFX or ADA, (3) enrolled patients with other diseases rather than IBD (psoriasis, rheumatoid arthritis), (4) only assessed the relationship between IFX or ADA TL and clinical outcomes but did not perform a receiver operating characteristic (ROC) curve analysis, or (5) did not present the specificity and sensitivity values of the ROC curve analysis. This last criterion was defined so that we could infer the accuracy of the cutoff value (i.e., a cutoff value with a sensitivity and/or specificity of 50% would be no better at identifying true positives than flipping a coin). It was not applied to abstracts found on ECCO, UEGW, or DDW databases.

Study Selection and Data Collection Process

Studies were screened and selected by 2 reviewers. First, all titles and abstracts were read and the inclusion and exclusion criteria were applied. Second, the articles considered for inclusion after selection by title/abstract reading were read fully and the inclusion and exclusion criteria were applied again. The data collected from each study were: the type of study and location, number of patients enrolled, and the type of IBD, definitions of clinical outcomes, antidrug antibodies incidence, type of assay used to measure IFX/ADA and ATIs/ATAs serum levels, and the results from the ROC curve analysis (cutoff levels and specificity and sensitivity values), except for the studies obtained in ECCO, UEGW, or DDW databases. In these studies, we have only had access to the abstract. A quality assessment was performed using a qualitative classification of the risk of bias. We used a 4-item classification based on the Meta-analysis of Observational Studies in Epidemiology checklist.[21] The items were chosen based on the factors that can incorporate bias, i.e., inclusion and exclusion criteria, justification of the cohort (eligibility criteria, sources and methods of selecting participants, and the methods used to describe follow-up), the type of disease (if they pointed out whether the patients included had CD or UC), and the assay used to measure drug and antidrug antibody levels (Fig. 1).

RESULTS

Search and Study Selection

A total of 1237 articles were identified with our query (Fig. 2). Of these, 1160 were excluded by title and/or abstract alone, mainly because they did not study the association between IFX or ADA TL and clinical outcomes. Therefore, 77 articles were considered for full text analysis and after that 13 were included in our systematic review (Fig. 2). Two additional articles were included after searching those related to the 13 articles selected by query.[22,23] From the search on ECCO, UEGW, and DDW abstract databases, 417 abstracts were found but only 5 were included, according to the inclusion criteria previously defined (Fig. 3).

Description of Studies

Of the 20 studies included, all but one[24] were conducted in adult patients. One study[25] only involved patients with UC, 11 studies[6,11,22,23,26–33] only encompassed patients with CD and 7 studies pertained to patients with either UC or CD.[8,24,34–38] Fifteen of the 19 studies involved IFX maintenance therapy[6,8,22,24–28,30–35,37] (Table 1), whereas the other 4 involved ADA maintenance therapy (Table 2).[11,23,29,36] One study encompassed patients from both regimens, IFX and ADA maintenance therapy.[38] Seven studies did not report information about the incidence of ATIs,[25,29,31–33,38,39] and only 6 performed an ROC curve analysis to find a cutoff value for ATI[22,32,34,35,37] or ATA[11] levels.

In 6 studies, the clinical outcome was "clinical remission"[8,11,22,24,28,36] usually assessed by the Harvey-Bradshaw Index–Mayo score and/or C-reactive protein (CRP) levels. In 4 studies, the outcome was "loss of response,"[26,32,35,37] defined as an initial good clinical response to IFX induction treatment followed by a loss of clinical response to IFX during maintenance treatment

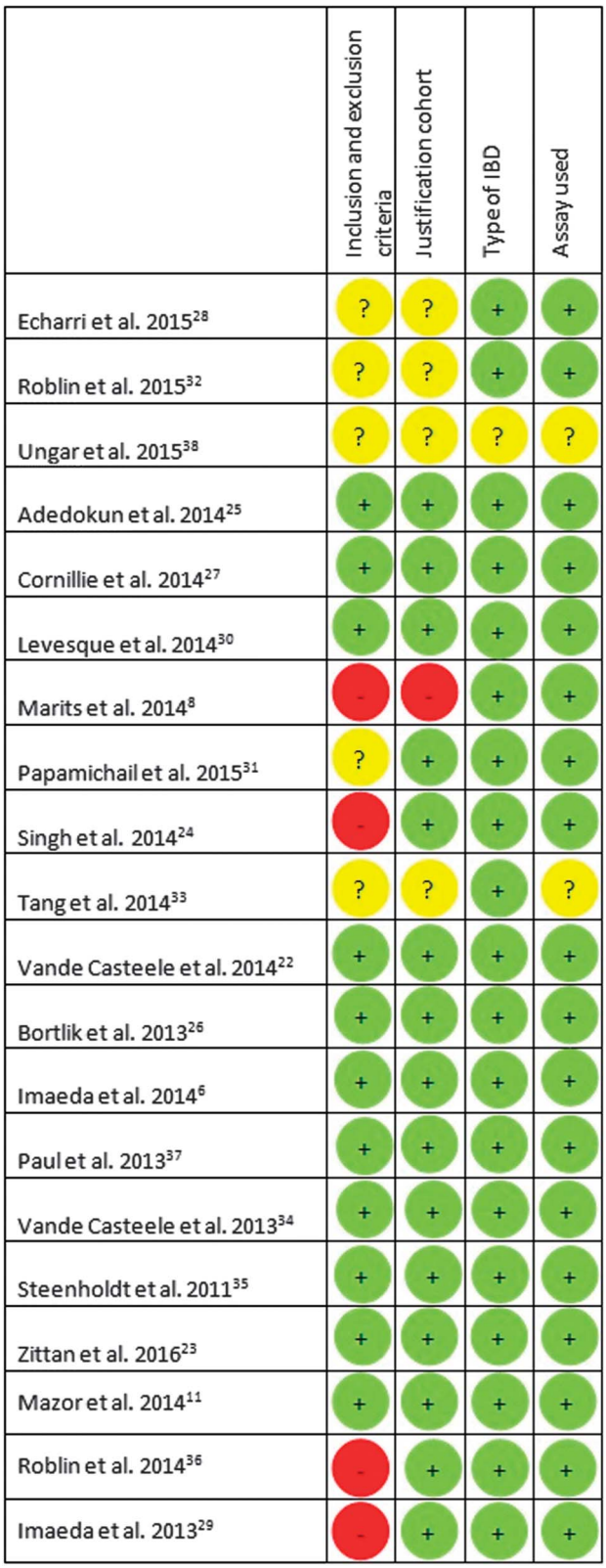

FIGURE 1. Summary of risk of bias.

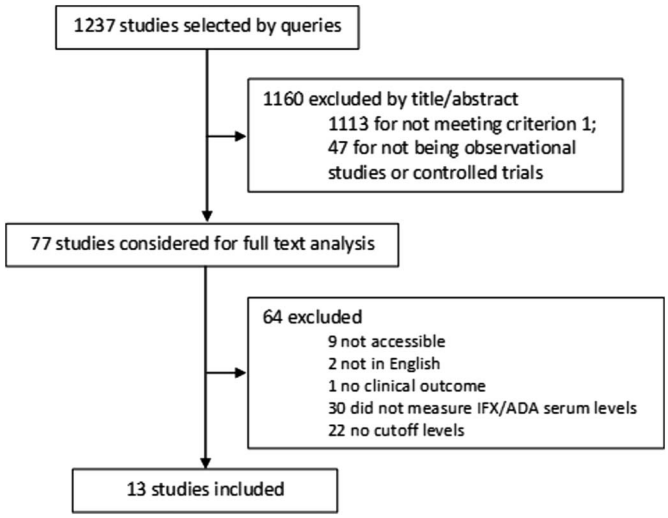

FIGURE 2. Data collection process.

leading to discontinuation of the drug. For Adedokun et al,[25] the endpoint was the "clinical response" defined as a decrease from the baseline in the total Mayo score of ≥3 points and at least 30%, and a decrease in the subscore for rectal bleeding of ≥1 or an absolute subscore for rectal bleeding of 0 or 1. For Levesque et al,[30] there were 2 endpoints which were an "increase in CD activity index ≥70" and an "increase in CRP ≥ 5 mg/L." Imaeda et al[6] defined 2 endpoints for IFX, including "mucosa healing," meaning an endoscopic score of 0 or 1, and "CRP ≤ 0.3 mg/L," whereas for ADA,[29] they only used "CRP ≤0.3 mg/L." Four more studies defined "mucosa healing" as the endpoint of interest.[23,31,33,38] Cornillie et al[27] defined clinical outcome as a "sustained response at week 54," which was expressed as clinical remission based on the relevant disease activity index at week 54, in the absence of any dose intensification during IFX maintenance therapy. Paul et al[37] also defined 2 endpoints: "loss of response" and "absence of clinical remission." Vande Casteele et al[34] described 3 endpoints which were "ATI formation," "IFX discontinuation," and "unsuccessful intervention." The intervention (change in therapy) was considered successful if, at the second infusion after the intervention, the symptoms had disappeared and CRP, if elevated before the intervention, had

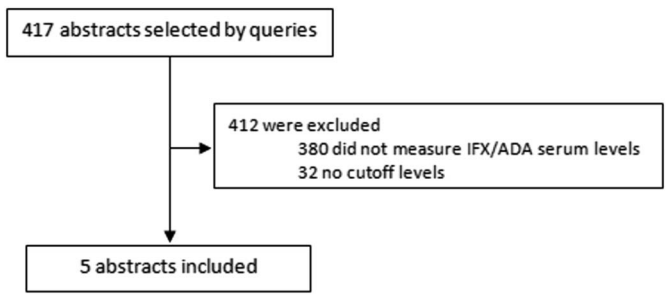

FIGURE 3. Data collection process.

TABLE 1. IFX Trough Levels and Antidrug Antibodies Cutoff, Methodology and Clinical Outcomes

Authors	Study Design	Population	Regimen	Country	Time Point
Echarri et al[28]	—	36 Adults with CD	IFX	Spain	W0, W6, W14, W30
Roblin et al[32]	Prospective cohort	119 Adults with CD	IFX	France	Trough level
Ungar et al[38]	Retrospective cross-sectional	78 Adults with IBD	IFX	Israel	No data
Adedokun et al, 2014[25]	Observational (post-hoc ACT1-2)	454 Adults with UC	IFX; induction regimen followed by maintenance therapy	globally	W8 W30 W54
Cornillie et al[27]	Observational (analyses of ACCENT I)	573 Adults with CD	IFX; induction regimen followed by maintenance therapy	North America, Europe, Israel	W14
Levesque et al[30]	Prospective cohort	327 Adults with CD	IFX; maintenance therapy	Canada	W8
Marits et al[8]	Retrospective	63 Adults with CD, 15 adults with UC, 1 adult with U-IBD	IFX	Sweden	Just before next infusion
Papamichail et al, 2015[31]	Retrospective	101 adults with CD	IFX	Belgium	W 0, 2, 6, 14
Singh et al[24]	Prospective cohort	58 pediatric patients (<21 years) with CD and UC	IFX; induction regimen followed by maintenance therapy	USA	W14
Tang et al[33]	No	15 adults with CD	IFX	China	No data
Vande Casteele et al[22]	Observational	483 adults with CD	IFX; maintenance therapy	Belgium Canada	No data
Bortlik et al[26]	Retrospective	84 adults with CD	IFX	Czech Republic	W14–22
Imaeda et al, 2014[6]	Prospective cohort	65 adults with CD	IFX; maintenance therapy	Japan	Just before next infusion
Paul et al[37]	Prospective cohort	103 adults with IBD	IFX; maintenance therapy	France	Just before next infusion
Vande Casteele et al[34]	Retrospective	64 adults with CD, 26 adults with UC	IFX	Belgium	Just before next infusion
Steenholdt et al[35]	Retrospective	85 adults with CD, 21 adults with UC	IFX	Denmark	Just before next infusion

	Drug			Antidrug antibodies				
Authors	Method	Cutoff, μg/mL	Spec/Sens, %	Method	Incidence, n (%)	Cutoff	Spec/Sens, %	Endpoint
Echarri et al[28]	ELISA	>3 (w6)	No	ELISA	No (26)	No	No	Good response and sustained remission
Roblin et al[32]	ELISA (commercial kit, Theradiag)	<2	No	ELISA	No	>20 ng/mL	No	Loss of response
Ungar et al[38]	No	>5	85/—	No	No	No	No	Mucosa healing

TABLE 1 (*Continued*)

	Drug			Antidrug antibodies				
Authors	Method	Cutoff, μg/mL	Spec/Sens, %	Method	Incidence, n (%)	Cutoff	Spec/Sens, %	Endpoint
Adedokun et al, 2014[25]	Classic ELISA	>41 (w8) >3.7 (w30) >1.7 (w54)	62/63 (w8) 71/65 (w30) 64/89 (w54)	Bridging ELISA	No	No	No	Clinical response
Cornillie et al[27]	Classic ELISA	≥3.5	78/64	Bridging ELISA	ATI+ = 2 (9)	No	No	Sustained response at w54
Levesque et al[30]	HMSA (commercial kit, Prometheus Laboratories)	≤2.8–4.6 (a) ≤2.7–2.8 (b)	68/61 (a) 74/64 (b)	HMSA (commercial kit)	ATI+ = 57 (18)	No	No	(a) Increased CDAI ≥70; (b) Increased CRP ≥5 mg/L
Marits et al[8]	Classic ELISA	>4.1 (CD)	44/87	Inhibition ELISA	ATI+ = 22 (79); ATI transient = 4 (18)	No	No	Remission (HBI-Mayo and CRP)
Papamichail et al, 2015[31]	ELISA	>22.5 (w2) >12.8 (w6)	No	No	No	No	No	Short-term mucosa healing
Singh et al[24]	Classic ELISA and HMSA (Prometheus Laboratories)	≥5 ≥7	85/50 100/33	Bridging ELISA and HMSA (Prometheus Laboratories)	No (10 at w14), no (26 at w54)	No	No	Week 54 persistent remission
Tang et al[33]	No	>4.87	77/88	No	No	No	No	Mucosa healing
Vande Casteele et al[22]	HMSA (commercial kit)	>2.79	77.6/52.5	HMSA (commercial kit)	23.7% (IFX−/ATI− = 6.5%; IFX +/ATI− = 69.8%; IFX−/ ATI+ = 16.4%; IFX+/ATI+ = 7.3%)	< 3.15 U/mL	87.4/38.0	Remission (CRP ≤ 5 mg/L)
Bortlik et al[26]	Classic ELISA (Q-INFLIXI, Matriks Biotek)	<3	62/70	Bridging ELISA (commercial kit)	ATI+ = 14 (17), ATI− = 24 (28), ATI inconclusive = 46 (55)	No	No	Loss of response at 1 year using IFX
Imaeda et al, 2014[6]	Classic ELISA	>4 (a) >0.6 (b)	70/71 (a) 62/73 (b)	ELISA (+acid dissociation and immunoaffinity crhomatography)	No	No	No	(a) Mucosa healing (b) CRP < 0.3 mg/L
Paul et al[37]	ELISA (commercial kit, Theradiag)	<2 (a)	82.3/76	Bridging ELISA (commercial kit)	ATI+ = 34 (32.8), CD = 25.4%, UC = 41.5%,[a] ATI+ = 65.3%[b]	>200 ng/mL (b)	93.5/22.0	(a) Absence of clinical remission (b) Loss of response.

TABLE 1 (*Continued*)

Authors	Drug			Antidrug antibodies				Endpoint
	Method	Cutoff, μg/mL	Spec/Sens, %	Method	Incidence, n (%)	Cutoff	Spec/Sens, %	
Vande Casteele et al[34]	Classic ELISA	<13 (w6) (a) <2.2 (w14) (a) <2.2 (w14) (c)	81/72 94/79 74/82	HMSA	ATI+ = 53 (59), Transient ATI = 15 (28), Sustained ATI = 38 (72)	>9.1 U/mL (b)	82/65	(a) ATI formation (b) Unsuccessful Intervention (c) IFX discontinuation.
Steenholdt et al[35]	Fluid-phase RIA	<0.5 (CD) <0.8 (UC)	85/86 (CD) 100/75 (UC)	Fluid-phase RIA	ATI+ = 35 (33.3)	≥10 U/mL (CD and UC)	90/81 (CD) 100/80 (UC)	Loss of response

[a]Cutoff for ATI positivity > 10 ng/mL.
[b]Cutoff for ATI positivity > 5 ng/mL.
CDAI, CD activity index; HBI, Harvey-Bradshaw index; sens, sensitivity; spec, specificity; U-IBD, unclassified inflammatory bowel disease.

decreased by >50% than the value at the time of loss of clinical response. Quality assessment was limited in those cases to which we only had access to the abstract.[28,31–33,38] Taking into consideration the other studies, all but 4 had suitable inclusion and exclusion criteria,[8,24,29,36] and all papers indicated the type of IBD and the assay used to measured drug and antidrug antibody levels.

Assays Used to Measure Drug and Antidrug Antibody Levels

One aspect that should be taken into consideration when analyzing drug TL and antidrug antibody levels is the assay used to measure them. All but 3 of the included works measured IFX or ADA TL using classic enzyme-linked immunosorbent assay (ELISA).[6,8,11,24–29,31,32,34,36,37] Zittan et al,[23] Levesque et al,[30] and Vande Casteele et al[22] used a homogeneous mobility shift assay (HMSA), whereas Steenholdt et al[35] used a fluid-phase radioimmunoassay (fluid-phase RIA). Singh et al[24] tested 2 methodologies, the classic ELISA and HMSA.

Regarding antidrug antibody measurements, 7 studies used bridging ELISA, either via home-made assays or commercial kits.[25–28,32,36,37] Mazor et al[11] applied an adaptation of the anti-human lambda chain-based ELISA. In 2 studies by Imaeda et al, ATI[6] and ATA[29] levels were also measured with ELISA, but samples were previously treated with acid in order to dissociate immune complexes. Other methods were used, namely HMSA,[23] fluid-phase RIA,[35] and inhibition ELISA.[8] Singh et al[24] tested 2 methodologies, bridging ELISA and HMSA. Figure 4 displays all methodologies used.

Infliximab Levels

By Week of Measurement

Of the 16 IFX studies, 7 specified the time point measurement[24–27,30]: one measured drug levels at week 2,[31] 2 at week 6,[28,31] 2 at week 8,[25,30] 3 at week 14,[24,26,27] 1 at week 22,[26] and 1[25] also measured IFX levels at weeks 30 and 54. Others only indicated that measurements were made before each infusion, thus representing drug TL.[6,8,11,29,34,35,37]

In Papamichaiel et al,[31] 2 cutoff levels were proposed (Table 1), both correlating with short-term mucosa healing, but after multiple logistic regression analysis, only IFX levels >12.8 μg/mL at week 6 were retained as an independent factor to predict short-term mucosa healing (OR: 3.6, $P = 0.004$). Echarri et al[28] presented a largely different cutoff level for the same time point. They suggest that IFX levels >3 μg/mL at week 6 had a positive-predictive value for "good response and sustained remission" of >90%. Adedokun et al[25] showed that IFX levels >41 μg/mL at week 8 correlated with clinical response with a specificity of 62% and a sensitivity of 63% (Table 1). The median serum IFX concentration was significantly higher at week 8 in patients with clinical response or mucosal healing during induction than those not achieving these endpoints. Levesque et al[30] found a different cutoff: a mean IFX trough concentration <3 μg/mL at week 8 was

TABLE 2. ADA Trough Levels and Antidrug Antibodies Cutoff, Methodology and Clinical Outcomes

						Drug		
Authors	Study Design	Population	Regimen	Country	Time point	Method	Cutoff, μg/mL	Spec/Sens, %
Zittan et al, 2016[23]	Observational	60 Adults with CD	ADA	Canada	—	HMSA (commercial kit, Prometheus Laboratories)	8.14	76.0/91.4
Ungar et al[38]	Retrospective, cross-sectional	67 Adults with IBD	ADA	Israel	—	—	>7.1	85/—
Mazor et al[11]	Observational, cross-sectional	71 Adults with CD	ADA	Israel	Just before next infusion	Classic ELISA	>5.85 (a)	70.6/68
Roblin et al[36]	Observational cross-sectional	40 Adults with IBD	ADA; maintenance therapy	France	W22	ELISA (commercial kit, Theradiag)	<4.9 (a) >4.85 (b)	85/66 67/81
Imaeda et al, 2014[29]	Prospective cohort	40 Adults with CD	ADA; maintenance therapy	Japan	Just before next infusion	Classic ELISA	>5.9	92/67

	Antidrug antibodies				
Authors	Method	Incidence, n (%)	Cutoff	Spec/Sens, %	Endpoint
Zittan et al, 2016[23]	HMSA (commercial kit, Prometheus Laboratories)	No (30.9)[a]	—	—	Mucosa healing
Ungar et al[38]	—	—	—	—	Mucosa healing
Mazor et al[11]	(adapted) Antihuman lambda chain-based ELISA	No (30.5 samples)[b] no (12.7 samples)[c]	≥ 3 μg/mL (b)	98/20.6	(a) Remission; (b) Active disease
Roblin et al[36]	Bridging ELISA (commercial kit)	9 (22.5)	No	No	(a) Absence of mucosa healing (b) Clinical remission
Imaeda et al, 2014[29]	ELISA (+acid dissociation)	35 (23)	No	No	CRP ≤ 0.3 mg/dL

[a]Cutoff for ATA positivity >1 U/mL.
[b]Cutoff for ATA positivity >1.5 μg/mL-eq.
[c]Cutoff for ATA positivity >3 μg/mL-eq.

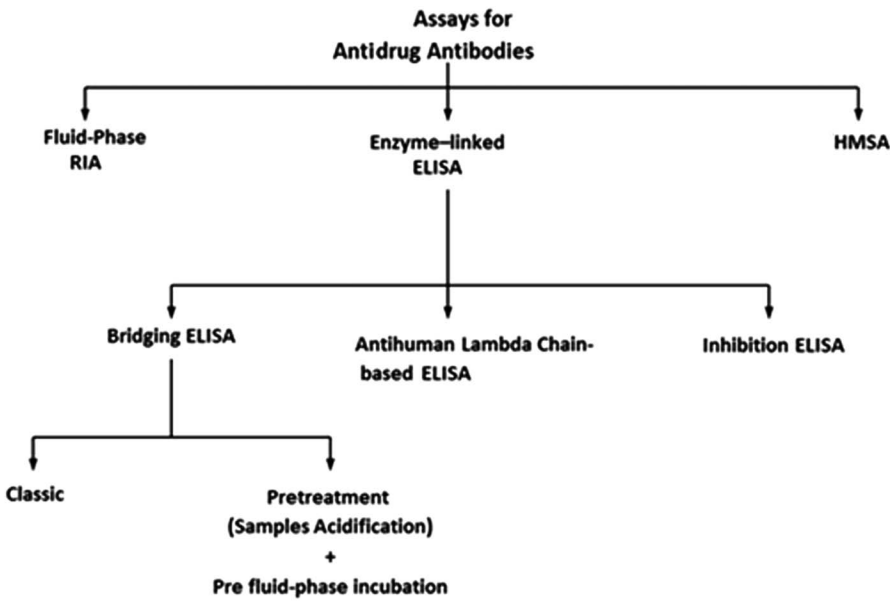

FIGURE 4. Methodologies for antidrug antibodies quantification.

significantly associated with a ≥70-point increase in the mean total CD activity index score between infusions ($P < 0.001$).

In measurements performed at weeks 14 and/or 22, cutoff values varied from <3[26] to ≥7 μg/mL[24] (Table 1). Patients with TL >3 μg/mL at weeks 14 and/or 22 had an approximately 66% lower likelihood to lose their response to IFX than those with subtherapeutic levels.[26] These findings are similar to data from the post-hoc analysis of the ACCENT I trial (A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen I),[27] which found that an IFX level >3.5 μg/mL at week 14 was a good predictor of sustained response at week 54. Patients with sustained response to scheduled maintenance IFX at 5 mg/kg had higher median IFX TL than those who lost response during the 54 weeks follow-up (4.0 versus 1.9 μg/mL, $P = 0.0331$). Adedokun et al[25] also measured IFX levels at weeks 30 and 54 and the levels, related with clinical response, were 3.7 and 1.7 μg/mL, respectively (Table 1). They suggested that more weight should be given to the threshold estimate at week 30 (3.7 μg/mL) because it was most representative of the steady-state trough concentration for both Active Ulcerative Colitis Trial studies. Therefore, patients with IFX TL >3.7 μg/mL at week 30 are more than twice as likely to have clinical response than patients with IFX TL <3.7 μg/mL.

Mucosa Healing

Imaeda et al[6] found that IFX TL >4 μg/mL was a good predictor of mucosa healing (Table 1). The authors also showed that the deterioration of the endoscopic findings was significantly associated with lower IFX TL. Two abstracts reported similar cutoff levels.[33,38] In both of them, IFX levels were significantly higher in the mucosal healing group than in patients with active disease (4.3 versus 1.7 μg/mL, $P = 0.0002$[38]).

Loss of Response

Steenholdt et al[35] showed that IFX TL <0.5 in CD and <0.8 in UC were good predictors of loss of response. IFX TL were significantly higher in both patients with CD and patients with UC who had maintained response to IFX compared with those who had lost response (median 2.8 μg/mL versus median 0 μg/mL, for CD; and 3.8 μg/mL versus 0 μg/mL for UC). Higher levels were identified by Bortlik et al[26] (<3 μg/mL) and Roblin et al[32] (<2 μg/mL).

Biomarkers

Imaeda et al[6] showed that IFX levels >0.6 μg/mL could predict normalized CRP levels (<3 mg/dL) with good sensitivity and specificity (Table 1). C-reactive protein levels were significantly higher in the nonmucosal healing group than in the mucosal healing group (0.09 versus 1.32 mg/dL). Levesque et al[30] showed that IFX concentrations <2.7 to 2.8 μg/mL predicted serum CRP levels >5 mg/L. Therefore, they suggested that a mean IFX trough concentration <3 μg/mL at week 8 was significantly associated with a higher probability for serum CRP concentrations >5 mg/L at that time point. In a study by Vande Casteele et al,[22] an IFX TL >2.79 μg/mL was considered to be a good predictor of CRP <5 mg/L, meaning that patients with IFX levels <2.79 μg/mL in a "current" sample were at higher risk of not achieving remission, defined as CRP <5 mg/L.

Adalimumab Levels

ADA information is sparse. Imaeda et al[29] evaluated 40 adults with CD and performed an ROC curve analysis to identify threshold levels of ADA that could predict normalized CRP levels (i.e., CRP ≤ 3 mg/dL). ADA levels >5.9 μg/mL predicted normalized CRP with high specificity (Table 2). Mazor et al[11] and

Roblin et al[36] conducted cross-sectional studies in patients taking ADA maintenance therapy; Mazor et al[11] enrolled patients with CD and Roblin et al[36] enrolled patients with CD or UC. In the study by Mazor et al, ADA TL >5.85 μg/mL predicted remission with a specificity and sensitivity of 70.6% and 68.0%, respectively. Roblin et al[36] showed that ADA serum concentrations <4.9 μg/mL predicted an absence of mucosa healing. The median ADA TL was significantly higher in cases of mucosa healing (6.5 versus 4.2 μg/mL in those without mucosa healing; $P < 0.005$). Moreover, serum levels higher than 4.85 μg/mL predicted clinical remission, defined as CD activity index <150 points or total Mayo score <3 (Table 2). Higher ADA TL were found in the work by Zittan et al (14.7 μg/mL in the mucosa healing group, versus 3.4 μg/mL in the non-MH group, $P = 6.25 \times 10^{-5}$).[23] Furthermore, Zittan et al suggested that ADA TL <8.14 μg/mL predicted MH with high sensitivity (Table 2). In the work by Ungar et al,[38] ADA levels >7.1 μg/mL identified patients with mucosa healing with 85% specificity. He also found that the association between higher levels of ADA and increased rate of mucosa healing reached a plateau at 12 μg/mL.

Incidence of ATIs and ATAs

Antidrug antibodies are described as the main cause of loss of response to biologic drugs over time. However, the incidence of antidrug antibodies varies significantly between studies. Taking into consideration those included in this systematic review, the ATI incidence varied from 9%[27] to 63.5%[37] (Table 1). In Bortlik et al,[26] 17% of the patients had ATIs but 55% were considered inconclusive. Marits et al[8] reported 22 out of 28 patients with ATIs, wherein 18% of them were ATI transient, meaning that patients presented with ATIs in their serum which at some point disappeared. The same was reported by Vande Casteele et al,[34] where 15 of the 53 patients considered with ATIs were transient (Table 1). Vande Casteele et al[22] reported an ATI incidence of 23.7%, and the authors were able to distinguish 4 groups of patients based on ATI and IFX status (Table 1). Paul et al[37] showed a global incidence of ATIs of 32.8%, considering a cutoff for ATIs of 10 ng/mL; with a cutoff of 5 ng/mL, the incidence was 63.5%. In the pediatric setting, 10% of the patients had ATIs in their blood at week 14, but the incidence increased to 16% at week 54.[24]

Although ADA is a fully human antibody, some patients develop ATAs. Imaeda et al[29] described a 23% incidence of ATAs. Roblin et al[36] found a similar value (22.5%). In the cross-sectional study from Mazor et al,[11] 12.7% of the samples had ATA levels ≥3 μg/mL; when a cutoff of ≥1.5 μg/mL was established, the incidence rose to 30.5% (Table 2). Zittan et al[23] described an ATA incidence of 30.9%, using a cutoff of >1 U/mL.

Cutoff Levels of ATIs and ATAs

Only 5 studies[11,22,34,35,37] performed an ROC curve analysis to identify threshold levels for antidrug antibodies. Steenholdt et al[35] reported that ATI levels, measured with fluid-phase RIA, >10 U/mL in patients with CD predicted "loss of clinical response" with a specificity of 90% and sensitivity of 81%. In the subgroup of patients with UC, the specificity was higher (Table 1). ATI were significantly lower in both patients with CD and patients with UC who had maintained response to IFX compared with those who had lost response (median 0 U/mL versus median 35 U/mL for CD, and median 0 U/mL versus median 85 U/mL for UC).

Paul et al[37] also performed an ROC curve analysis using "loss of response" as the target clinical outcome. The authors suggested that ATI levels >200 ng/mL, assessed by the ELISA assay, predicted loss of response with a high specificity but with a low sensitivity (Table 1). A combined analysis was also performed on patients with CD with IFX levels <2 μg/mL and ATI levels <200 ng/mL. The ATIs predicted clinical remission with a high specificity and sensitivity (Table 1); patients with UC showed higher specificity (100%) but lower sensitivity (70%). The same analysis using "mucosa healing" as the clinical outcome was also supplied (Table 1). An ROC curve analysis for a threshold >9.1 U/mL at the time of loss of response predicted an "unsuccessful intervention" with a specificity of 82% and a sensitivity of 65%.[34] Therefore, patients having ATI TL >9.1 U/mL at the time of loss of response had a likelihood ratio of 3.6 for an unsuccessful intervention. It was also reported that patients with ATI levels <3.15 U/mL had a higher probability of being in remission.[21]

With regard to ADA, Mazor et al[11] suggested that a cutoff level ≥3 μg/mL, when using an adapted anti-human lambda chained-based ELISA assay, predicted active disease with high specificity but low sensitivity (Table 2). The authors showed a negative correlation between ADA drug levels and ATA levels and found that for patients with ATA levels ≥3 μg/mL-eq, the maximal ADA level was only 0.5 μg/mL.

DISCUSSION

The importance of measuring drug levels and antidrug antibody levels to adjust therapy is undisputable. The major hindrance to its implementation in daily clinical practice is the lack of a universally valid assay and the absence of a cutoff level clearly related with a clinical outcome. One cannot easily compare results from different studies, as they use distinct assays that have different limitations and lower limits of quantification.

Regarding the measurement of IFX levels, classic ELISA is the methodology most frequently used, but other methods are available, such as HMSA and fluid-phase RIA. Studies[40–42] that have compared performance of different methods to measure drug levels have concluded the same; there is a good qualitative correlation between different assays (e.g., IFX detection rates of 76% with ELISA and 82% with RIA[42]). Furthermore, in some cases, there is a good quantitative correlation (e.g., ELISA and RIA, $R^2 = 0.98$, $P = 0.001$[40]; ELISA and RIA, Pearson $r = 0.91$, $P < 0.0001$[41]) but not a perfect agreement on drug concentrations (e.g., maximum difference of 1.41 μg/mL between ELISA and RIA[40]), and this emphasizes the importance of

establishing different cutoff levels according to the methodology used. The threshold levels assessed by ROC curve analysis were quite different between the studies. This can be due to (1) different methodology (even using the same principle, such as bridging ELISA, home-made ELISA, and commercial kits), (2) different study design and sample characteristics, and/or (3) different endpoints. This heterogeneity justifies the obstacle to perform a meta-analysis. A systematic review and meta-analysis was recently published on this topic and suggested a cutoff level of 2 μg/mL to predict remission (RR = 2.9, 95% confidence interval, 1.8–4.7, $P < 0.001$), but there was a high statistical heterogeneity ($I^2 = 88\%$).[43] However, TL were always associated with a better clinical endpoint: clinical remission, mucosa healing, normalized CRP, or loss of response. Our review emphasizes the importance of measuring drug levels during maintenance therapy as well as in cases of loss of response, cases with persistent high levels of CRP, and when mucosal lesions are still present. In the induction phase, the only study reported did not show any advantage of measuring IFX at 2 weeks because this corresponded to the loading period and it was not possible to differentiate responders from nonresponders. However, at weeks 8, 14, and 30, the different studies found significant differences between responders and nonresponders, and one of these time periods should be chosen by clinicians for strategic therapeutic decisions, namely increasing drug dose or addition of 1 immunomodulatory drug. Active Ulcerative Colitis Trial subanalysis suggested week 30 is ideal and argued that this time corresponds to the steady state of the drug. Two studies (TAILORIX[44] and TAXIT[45]) have concluded that in maintenance phase, concentration-based dose adjustment was not superior to dose adjustment based on symptoms alone. However, TAXIT trial also showed that patients in the "clinically based dosing" group had more flares during the course of treatment than those in the "concentration-based dosing" group.

Overall, there is evidence for determining drug levels in weeks 6, 14, 22, 30, and 54. During maintenance, therapeutic drug monitoring should be considered in case of loss of response, mucosal ulceration, and elevated biomarkers, such as CRP and fecal calprotectin (Fig. 5).

Figure 6 shows how therapeutic drug monitoring may be used to highlight factors influencing loss of response. Two branches are schematized: for patients with loss of response and high levels of drug (pink branche) and for patients with loss of response and low drug levels (green branche). Pharmacodynamic, pharmacokinetic, and immunogenicity factors may be identified and help clinicians to handle therapeutic decisions.

All methodologies available (ELISA, HMSA, fluid-phase RIA) seem qualitatively equivalent, so either one can be used to monitor drug levels. However, the clinician should take into consideration that there are disagreements on IFX concentration between assays, therefore for each patient, drug levels should be always measured with the same assay. Concerning antidrug antibody levels, the variability among methods is more significant. Enzyme-linked immunosorbent assay (ELISA) is the methodology most frequently used; however, not all ELISAs use the same principle. A bridging ELISA, or double antigen ELISA, uses the drug, in this case, IFX or ADA as the captured antigen and as the detection antibody. Consequently, this method is susceptible to several limitations, namely false-positive results, caused by rheumatoid factors or activated complement fragments that cross-bind the drug's fragment crystallizable region. False-negative results are due to the assay's inability to detect monovalent immunoglobulin G4 (IgG4) and antidrug antibodies in the presence of the drug. This method was used by 6 of the studies included in this systematic review.[24–27,36,37] Since this assay has no sensitivity to detect antidrug antibodies in the presence of the drug, some of the studies did not measure antidrug antibodies if there were drug levels in the serum and considered those samples

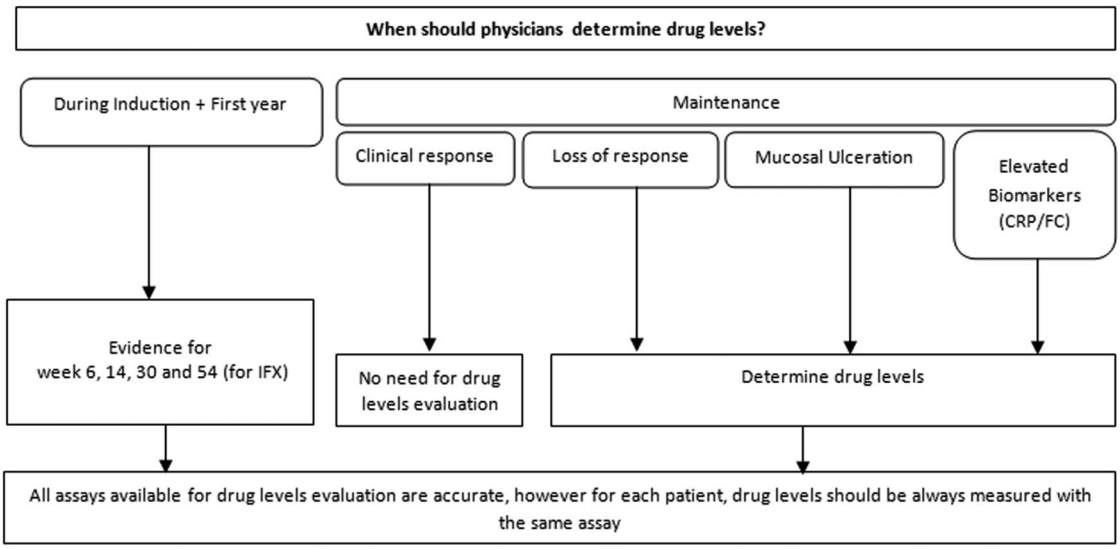

FIGURE 5. Time points for drug level determination.

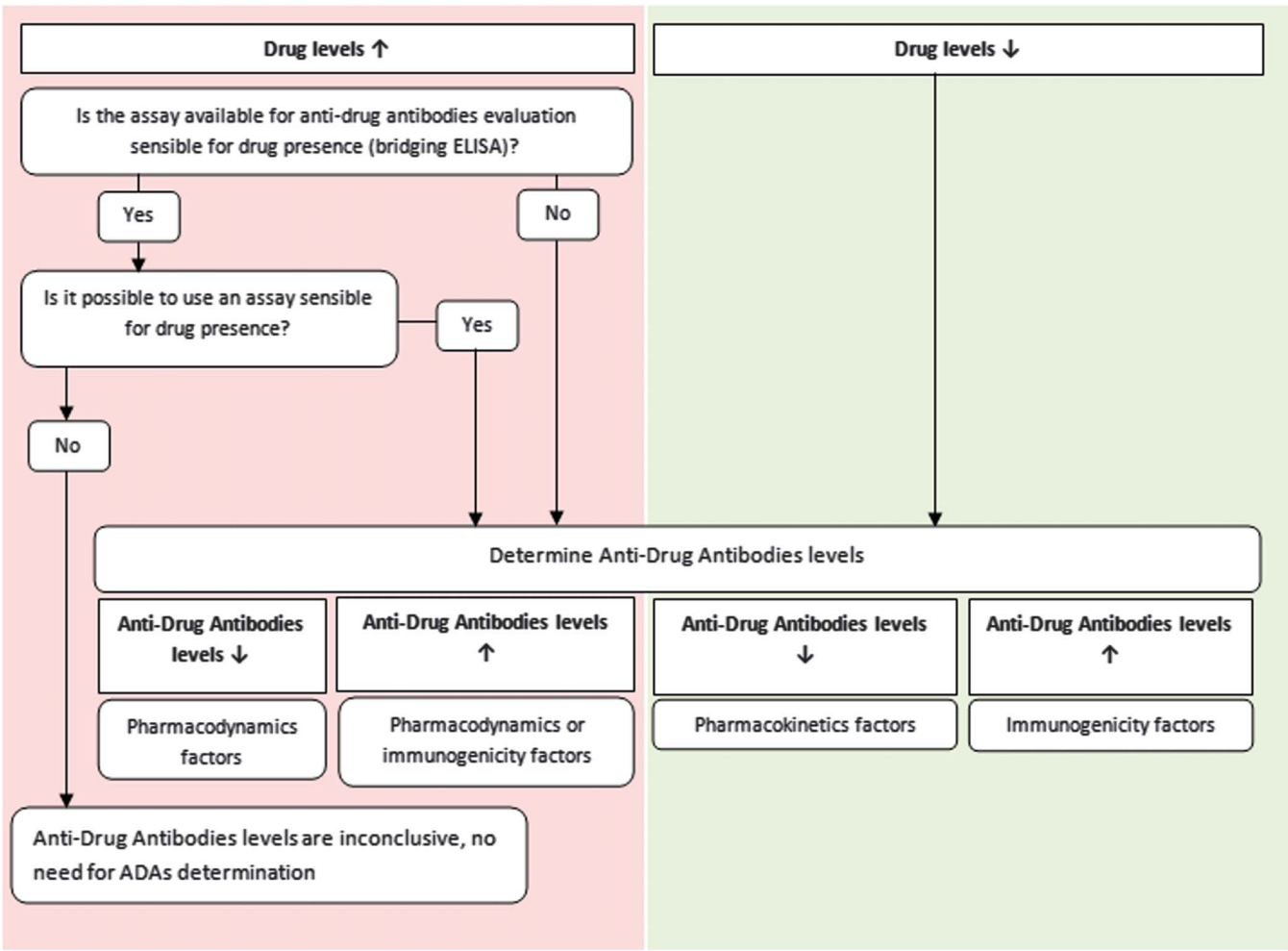

FIGURE 6. Therapeutic drug monitoring brought to light the factors influencing the loss of response.

as "ATI inconclusive." Cornillie et al[27] considered samples that had IFX levels >0.1 μg/mL as "ATI inconclusive," whereas Bortlik et al[26] only considered samples that had IFX levels >3 μg/mL as "ATI inconclusive." Given that half of the patients in clinical trials had the drug in their serum, the use of a bridging ELISA for anti-IFX detection may lead to serious bias. This must be taken into consideration when one tries to draw conclusions about the therapeutic importance of ATIs using bridging methodology.[35]

We should also keep in mind that study populations and study designs were different. Some included only patients with CD or UC, whereas others comprised both types of patients; some were prospective cohorts while others were cross-sectional studies or post-hoc analyses of controlled trials. These differences can explain why the incidence of antidrug antibodies was so varied between them, even when using the same assay. For example, both studies from Cornillie et al[27] and Paul et al[37] used a bridging ELISA to measure antidrug antibodies but the incidence of ATI positivity was 9% and 32.8%, respectively. This could be explained by the fact that the first study was a post-hoc analysis of the ACCENT I trial that enrolled 573 adult patients with CD, whereas the second was a prospective cohort with 103 adults with CD or UC.

Kopylov et al[46] developed a different ELISA method, anti-human lambda chain-based ELISA, to overcome the false-negative results associated with the presence of the drug. The authors took advantage of the fact that antidrug antibodies have a lambda light chain, whereas the drug has a kappa light chain, and they used an anti-lambda antibody as the detection antibody, ensuring that they were only measuring antidrug antibodies. Mazor et al[11] adapted this method to measure antibodies to ADA. Those authors described an incidence for ATA positivity of 30.5%, which showed the sensitivity of anti-lambda chain ELISA and its low rate of drug interference. However, in serum with high levels of a drug, even anti-lambda chain ELISA is not able to completely overcome drug interference.[47] Anti-lambda chain ELISA is also unable to detect anti-idiotypic antibodies, i.e., antibodies that recognize functional binding epitopes.[47]

One way of overcoming drug interference is to perform a prior acidic dissociation. Imaeda et al pretreated samples with

acid in both the IFX study and ADA study.[6,29] In a previous work, the authors showed the ability of this new method to detect ATIs in samples containing detectable levels of IFX, which proved to be more accurate than the bridging ELISA.[48] From a total of 58 samples, the methodology by Imaeda et al could detect an additional 14 positive samples, of which, by the bridging ELISA, 8 had been considered negative and 6 "inconclusive."

Three studies[24,30,34] used the HMSA to measure ATIs, an alternative assay to ELISA. The HMSA uses size exclusion high-performance liquid chromatography.[47] Although HMSA requires expensive equipment, the authors of those studies state many advantages, including the ability to overcome many potential artifacts encountered in the solid-phase ELISA, the ability to detect high and low affinity antibodies (low affinity antibodies may not be detected by ELISA due to multiple washing steps), the detection of all immunoglobulin isotypes and all IgG subclasses (including IgG4), and the fact that it is not affected by substances present in serum.[49] However, a different ATI incidence was reported by the 3 studies, which can be explained by differences in the study population and sample size (Table 1).

Another assay is able to bridge the gaps of the ELISA methodology. In fluid-phase radioimmunoassay (RIA), used by Steenholdt et al,[35] a radio-labeled antibody to detect and quantify the amount of antidrug antibodies is applied. It has proved to be more sensitive than ELISA, as it is able to detect antidrug antibodies in the presence of the drug and IgG4 isotype.[40,41] Moreover, fluid-phase RIA overcomes matrix effects encountered in solid-phase assays due to epitope masking via protein aggregation. The major limitation of RIA is the need for advanced laboratory facilities.[47]

Therefore, the differences in methodology, study design, and sample size and characteristics may also explain why the 4 studies with IFX[22,34,35,37] that performed an ROC curve analysis in order to find a cutoff level of antidrug antibodies related with a clinical outcome found different threshold levels. It is also not easy to compare the thresholds between studies because they used different units (U/mL; μg/mL; ng/mL) and defined different endpoints. A serious limitation of all of the studies was the inability to show whether or not antidrug antibodies were neutralizing.

It is important to address whether or not antidrug antibodies are functional, because we know that antidrug antibody detection in serum does not always correlate with loss of clinical response.[47,50] Moreover, sometimes the presence of antidrug antibodies may actually increase the half-life of the drug; if 1 or 2, but not more, antidrug antibodies bind to the drug, the complex will bind to Neonatal fragment crystallizable receptor and will escape elimination.[51] A study comparing different methodologies (ELISA, EIA, RGA, RIA) to measure antidrug antibody levels has been published and concluded that the ability to detect anti-ATIs is comparable with respect to basic analytical properties. ELISA and RIA showed a good correlation ($R^2 = 0.73$, $P = 0.03$), but the agreement was not so good, with a mean titer difference of −2400 (−5000 to 200), which can be partially explained by the inability of bridging ELISA to detect IgG4 antidrug antibodies. The authors suggest that clinicians should choose an assay where assessments take place in fluid phase and where all anti-IFX IgG isotypes are quantified.[40]

CONCLUSION

Currently, there is no doubt that drug levels correlate with clinical and endoscopic outcomes, and this knowledge is the basis of drug monitoring. Nevertheless, it can only be widely used in clinical practice when there is a consensus on the thresholds of drug and antidrug antibody levels that correlate with a specific clinical outcome, including either clinical remission or loss of response. Concerning drug level monitoring, any methodology is adequate but the data published by now is insufficient to come up with a cutoff level. With respect to antidrug antibody levels, assays have significantly different sensitivity, therefore it will be necessary to define a gold standard method or to establish different cutoff levels for different methodologies.

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Inflammatory Bowel Diseases

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Journal Article
1. Gudlaugsdottir S, van Dekken H, Stijnen T, et al. Prolonged use of proton pump inhibitors, CagA status, and the outcome of Helicobacter pylori gastritis. *J Clin Gastroenterol*. 2002;34:536-540.

Book Chapter
2. Tobin RW, Kimmey MB. Painful diseases of the gastrointestinal tract. In: Loeser JD, ed. *Bonica's Management of Pain*. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1269-1292.

Entire Book
3. Rohen JW, Yokochi C, Lütjen-Drecoll E. *Color Atlas of Anatomy: A Photographic Study of the Human Body*. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.

Software
4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention; 1994.

Online Journals
5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. ObstetGynecol [serial online]. January 1988;71:22-37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database
6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 2014. Updated March 29, 2014.

World Wide Web
7. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS Web site]. June 1, 2015. Available at: http://www.ama-assn.org/special/hiv/ethics,. Accessed July 26, 2015.

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